retraining. After rehabilitation the case patient also demonstrated movement patterns that more closely reflect the patterns of patients without knee pathology and these variables were substantially improved compared to her pre-operative levels. Her knee flexion excursion and knee flexion moment in the operated limb were nearly twice that of the TKA patients who did not undergo the specialized rehabilitation (Figure 1) and she demonstrated a 40% reduction in the adduction moment of her non-operated limb. Her knee excursion symmetry ratio (operated/non-operated limb) was 1.0, whereas the symmetry ratio was 0.7 for the other TKA group.

Table 1. Patient characteristics at all time points

	Pre-operative		Initial PT Evaluation		Discharge from PT	
	Case patient	TKA group (SD)	Case patient	TKA group (SD)	Case patient	TKA group (SD)
KOS (%)	37.14	50.28 (17.43)	32.86	55.9 (13.61)	98.60	79.02 (12.35)
TUG (s)	11.1	10.2 (2.8)	14.3	11.9 (3.5)	9.6	8.3 (1.9)
SCT (s)	23.4	20.3 (9.5)	36.4	26.8 (12.2)	13.1	13.8 (15.1)
6MW (ft)	1442	1487 (410)	1073	1319 (337)	1709	1733 (358)
Knee Flexion ROM	135	118 (14)	95	98 (15)	123	115 (11)
Quadriceps (N/BMI)	11.37	18.7 (7.5)	3.91	10.0 (4.3)	12.30	17 (6.7)

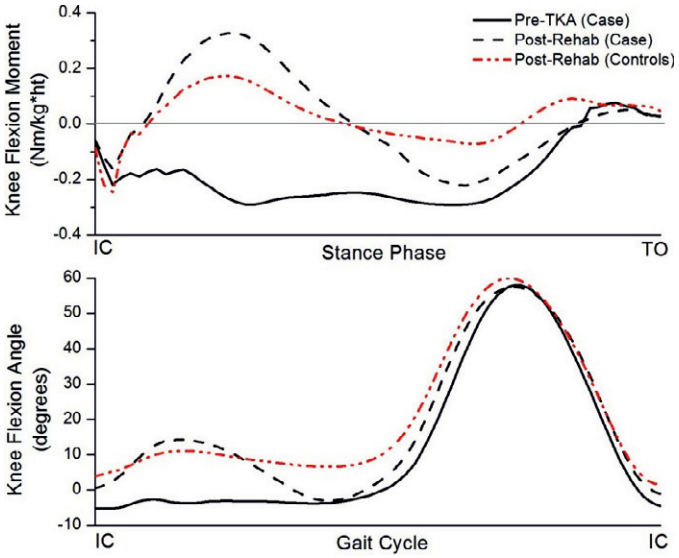

Fig. 1.

Conclusions: In this case study, the rehabilitation protocol that focused on improving limb symmetry and normalizing joint motion on the involved leg resulted in dramatic improvements in knee biomechanics and functional outcomes. This restoration of symmetrical and normalized movement patterns may have important implications on reducing the incidence of contralateral joint OA. Future research is warranted to investigate the efficacy of this program on a larger sample of patients after TKA.

Funding for this study was provided by NIH P20RR01645–08S2 and P20RR01645

194

FOOT CENTER OF PRESSURE MANIPULATION AND GAIT THERAPY INFLUENCE LOWER LIMB MUSCLE ACTIVATION IN PATIENTS WITH OSTEOARTHRITIS OF THE KNEE

Y. Goryachev[1], E. Debbi[1], A. Haim[1,2], N. Rozen[3], A. Wolf[1]. *[1]Technion-Israel Inst. of Technology, Haifa, Israel; [2]Sourasky Med. Ctr., Tel-Aviv, Israel; [3]Ha'Emek Med. Ctr., Afula, Israel*

Purpose: Foot center of pressure (COP) manipulation has been associated with improved gait patterns. The purpose of this study was to determine lower limb muscle activation changes in knee osteoarthritis patients, both immediately after COP manipulation and when COP manipulation was combined with continuous gait therapy (AposTherapy).
Methods: Fourteen females with medial compartment knee osteoarthritis underwent EMG analyses of key muscles of the leg. In the initial stage, trials were carried out at four COP positions. Following this, gait therapy was initiated for three months. The barefoot EMG was compared before and after therapy.
Results: The average EMG varied significantly with COP in at least one phase of stance in all examined muscles of the less symptomatic leg and in three muscles of the more symptomatic leg. After training, a significant increase in average EMG was observed in most muscles. Most muscles of the less symptomatic leg showed significantly increased peak EMG. Activity duration was shorter for all muscles of the less symptomatic leg (significant in the lateral gastrocnemius) and three muscles of the more symptomatic leg (significant in the biceps femoris). These results were associated with reduced pain and increased function.
Conclusions: COP manipulation influences the muscle activation patterns of the leg in patients with knee osteoarthritis. When combined with a therapy program, muscle activity increases and activity duration decreases.

Bone Biology

195

LOAD-INDUCED SUBCHONDRAL BONE THICKENING IN MICE WITH OR WITHOUT ARTICULAR CARTILAGE LESIONS

B. Poulet, L. Saxon, O. Barker, R. De Souza, A.A. Pitsillides. *The Royal Vet. Coll., London, United Kingdom*

Purpose: Subchondral bone remodelling is an important characteristic of osteoarthritis (OA) in humans and in animal models. However, the relationship between SCB changes and articular cartilage (AC) degeneration remains controversial: do they precede or follow AC lesions? To begin answering this question, we analysed changes in SCB thickness in a mouse model of knee joint loading, in which localised AC lesions are generated in the lateral femur where it becomes closely opposed to the tibia during loading.
Methods: Right knees of 8 week-old CBA mice were loaded 3 times each week for 2 (± 3 weeks of normal use with no loads applied) or 5 weeks at a magnitude of 9N as described previously[1+2]. Micro-CT scanning was performed on left (non-loaded) and right (loaded) knee joints and SCB thickness measured in the posterior half of each condyle using CTAn software, and in order to precisely define their spatial relationship to lesions in the lateral femur, in 0.1 mm sections within this posterior half. Paired t-test was used for statistical analysis.
Results: SCB thickness was increased in the regions of the lateral femur which were closely associated with load-induced AC lesions, and no changes were noted in regions remote from these lesions. Joints loaded for 5 weeks showed most obvious thickening in SCB. In addition, SCB thickness was increased in the most posterior region of the lateral tibia, where no AC lesions were induced by the application of mechanical loading, but which was directly in contact with the lateral femur AC during loading. This SCB thickening was again most prominent after 5 weeks of loading.
Conclusions: We have described focal thickening of SCB associated with load-induced AC lesion formation in the lateral femur, as well as thickening of SCB in areas exposed to direct mechanical loads (without cartilage lesions) in the lateral tibia. This indicates that SCB changes can be induced by loading independently of AC lesions, and that altered load distribution associated with the presence of AC lesions acts to enhance load-induced SCB thickening. These data suggest that SCB thickening is due to altered mechanical loads in OA joints.

196

BLOOD PERFUSION AND BONE FORMATION BEFORE AND AFTER MINIMALLY INVASIVE PERIACETABULAR OSTEOTOMY ANALYSED WITH PET COMBINED WITH CT

I. Mechlenburg[1], F. Hermansen[2], T. Thillemann[1], K. Søballe[1]. *[1]Aarhus Univ. Hosp., Aarhus, Denmark; [2]PET-Ctr. Aarhus Univ. Hosp., Aarhus, Denmark*

Purpose: A new minimally invasive technique for periacetabular osteotomy (PAO) has been developed in our institution. The osteotomized acetabular fragment is reoriented three dimensionally in order to achieve a better acetabular coverage. Bone healing is believed to be completed eight weeks after surgery and from that time, the patients are allowed to fully weight-bear on the operated leg. Sufficient blood perfusion is held to be essential to successful bone healing after PAO. It is never examined in vivo how blood perfusion to the acetabular fragment is affected by

PAO and whether perfusion contributes to new bone formation in the acetabular fragment. The purpose of this study was to quantify blood perfusion and bone formation before and after PAO analysed by Positron Emission Tomography (PET) combined with Computed Tomography (CT).

Methods: Twelve dysplastic patients (nine women) were included consecutively in the study all operated by the senior author. Median age was 33 (23–55) years. Initially, two patients were PET scanned in a pilot study to test our models for calculation of the physiological parameters. The following ten patients had their hip joints PET/CT scanned immediately before PAO and 3–4 weeks after. Due to patients moving on the scanner bed while scanning, data of sufficiently high quality was only available for six out of ten. [O-15]-water was used to quantify blood perfusion and [F-18]-fluoride was used to produce quantitative images interpreted as new bone formation in/around the acetabular fragment. The perfusion [ml blood/min/ml bone] was determined from a one-compartment model, with the parameters: K_1, k_2 and the delay. The fluoride-clearance per volume bone (K_i) [ml blood/min/ml bone] was determined by applying Patlak graphical analysis to the fluoride scan, fitting the data from 45 to 90 min.

Results: The blood perfusion on the operated acetabulum before surgery was 0.07±0.02 ml/min/ml, and after surgery 0.19±0.03 ml/min/ml ($p < 0.00$). Blood perfusion on the non-operated acetabulum was 0.07±0.02 ml/min/ml before PAO and 0.07±0.02 ml/min/ml after surgery ($p = 0.47$).

The fluoride-clearance per volume bone on the operated acetabulum was 0.02±0.01 ml/min/ml preoperatively, and 0.06±0.01 ml/min/ml postoperatively ($p < 0.00$). Fluoride-clearance on the non-operated acetabulum was 0.01±0.01 ml/min/ml before PAO and 0.02±0.01 ml/min/ml after PAO ($p = 0.49$).

Conclusions: Blood perfusion and new bone formation increased significantly in the acetabular fragment demonstrating that blood perfusion to the acetabular fragment is not critically compromised after minimally invasive PAO a.m. Soballe. Three to four weeks after PAO, bone formation in the acetabular fragment on the operated side had increased significantly. This is the first paper applying PET/CT to quantify blood perfusion and bone formation before and after PAO.

197
SMOOTHENING OF PERIARTICULAR BONE: BLOCKADE OF THE HEDGEHOG PATHWAY INHIBITS OSTEOPHYTE FORMATION IN ARTHRITIS

G. Ruiz-Heiland[1], A. Horn[1], P. Zerr[1], W. Hofstetter[2], W. Baum[1], M. Stock[1], J. Distler[1], F. Nimmerjahn[1], G. Schett[1], J. Zwerina[3]. *[1]Univ. of Erlangen, Erlangen, Germany; [2]Univ. of Bern, Bern, Switzerland; [3]Hanusch Hosp., Vienna, Austria*

Purpose: Osteophyte formation is a common phenomenon in arthritis. Bone formation by endochondral ossification is considered a key pathophysiologic process to form osteophytes. We hypothesized that inhibition of Smoothened (Smo), a key component of the hedgehog pathway inhibits osteophyte formation as the hedgehog pathway mediates endochondral ossification.

Methods: We induced arthritis in 8 weeks old C57/BL6 mice by serum transfer (KxBN model). Mice were then treated by daily administration of either vehicle or LDE 223, a specific small molecule inhibitor for Smo, over 2 weeks starting at the onset of disease. Clinical course of arthritis, histological and molecular changes of bone in the affected joints as well as systemic bone changes were assessed.

Results: Serum transfer induced arthritis led to severe osteophyte formation within 2 weeks of onset. Blockade of Smo inhibited hedgehog signaling in vivo and also significantly inhibited osteophyte formation, whereas the clinical and histopathologic signs of arthritis were not affected. Also, systemic bone mass did not change. Smo inhibitor particularly blocked the formation of hypertrophic chondrocytes and collagen type X expression.

Conclusions: Our data indicate that blockade of hedgehog signaling by targeting Smo specifically inhibits osteophyte formation in arthritis without affecting inflammation and without eliciting bone destruction at the local and systemic level. Blockade of SMO may thus be considered as a strategy to specifically influence the periosteal bone response in arthritis associated with bone apposition.

Cartilage Biology & Biochemistry

198
TNFa INDUCES SIRT1 CLEAVAGE IN HUMAN OSTEOARTHRITIC CHONDROCYTES

M. Dvir-Ginzberg[1], O. Gabay[2], H. Oppenhiemer[1], H. Meir[1], V. Gagarina[2], E. Lee[2], A. Haze[1], L. Kandel[3], M. Liebergall[3]. *[1]Hebrew Univ. of Jerusalem, Jerusalem, Israel; [2]NIAMS, Bethesda, MD, USA; [3]Hadassah Mount Scopus Hosp., Jerusalem, Israel*

Purpose: Osteoarthritis (OA) is a common degenerative joint disease of articular cartilage (AC) characterized by a disrupted homeostasis of extracellular matrix (ECM) synthesis and breakdown. It is often thought that mechanical wear and tear of AC elicits OA pathology. However, increasing reports indicate that synovial inflammation occurs in OA, resulting in augmented levels of proinflammatory cytokines (mainly TNFa and IL-1b) within synovial fluid. Given that the NAD-dependent protein deacetylase SirT1 promotes cartilage-type ECM expression and chondrocyte survival, we postulate its function is altered in chondrocytes exposed to proinflammatory cytokines, as TNFa.

Methods: TNFa-treated and untreated human osteoarthritic chondrocytes were analyzed for cartilage-specific gene expression, SirT1 activity and ChIP analyses at the collagen 2a1 enhancer site. Human chondrocytes transfected with an N-terminal Flag-tag SirT1 expression vector, were treated with or without TNFa and analyzed by immunoblot for the presence of SirT1. Protein extracts were immunoprecipitated for SirT1 following TNFa-treatment and analyzed via mass-spectroscopy and Edman sequencing. In-vitro analysis of SirT1 activity and cleavage was assayed in the presence of active Cathepsin B.Confocal images of SirT1 monitored its subcellular trafficking following TNFa stimulation. Co-immunofluorescent staining and confocal visualization was carried out for Cathepsin B, mitochondrial Cox IV and Lysosome-associated membrane protein I (LAMP-I) together with SirT1. Human chondrocyte were tested for apoptosis via FACS analysis for Annexin V and immunoblotting for active caspase 3 and 8. TNFa treated mitochondrial extracts were obtained and immunopreciptated to detect the presence of cleaved-SirT1. Finally human osteoarthritic and normal samples were analyzed for the presence of active Cathepsin B, MMP13 and cleaved-SirT1.

Results: TNFa-treated chondrocytes had impaired SirT1 enzymatic activity and displayed full-length SirT1 protein (110kDa, FL-SirT1) and a smaller 75kDa SirT1 fragment (i.e 75kDa SirT1). 75kDa SirT1 was generated via Cathepsin B-mediated cleavage at residue 533, following TNFa stimulation. Confocal images revealed that 75kDa SirT1 was exported to the cytoplasm and colocalized with mitochondrial membrane protein Cox IV, following TNFa stimulation. Prohibiting nuclear export of 75kDa SirT1 via Leptomycine B or reducing its protein levels in the presence of TNFa, led to a 10-fold increase in apoptotic chondrocytes. Finally, Cathepsin B, responsible for 75kDa SirT1 generation, was found elevated in TNFa-treated and OA-derived chondrocytes vs. untreated and normal chondrocytes, respectively. As an additional proof of principle, we show that normal human chondrocytes exposed to synovial fluid derived from OA patients generate 75kDa SirT1 fragment.

Conclusion: These data indicate that TNFa, a cytokine that mediates joint inflammation in OA, induces Cathepsin B-mediated cleavage of SirT1, resulting in a cytoplasmic 75kDa SirT1 fragment with impaired enzymatic activity. The impaired enzymatic activity of 75kDa SirT1 correlates with reduced cartilage-ECM gene expression evident in TNFa treated chondrocytes. In parallel, our data show that the stable 75kDa SirT1 fragment promotes chondrocyte survival when exposed to TNFa.

199
THE ROLE OF THE PROGRESSIVE ANKYLOSIS PROTEIN (ANK) IN OSTEOARTHRITIS

K. Campbell, S. Hadley, T. Minashima, T. Kirsch. *NYU Sch. of Med., New York, NY, USA*

Purpose: Currently there are no treatments available for osteoarthritis (OA). In order to establish new therapeutic strategies for the treatment of OA, a better understanding of the cellular and molecular changes during OA progression is required. The progressive ankylosis protein (ANK) is a